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TECHNICAL MANUSCRIPT 358

AGAR-GEL PRECIPITIN-INHIBITION TEST FOR COCCIDIOIDOMYCOSIS: I. Preliminary Evaluation of the Complement-Fixation and Agar-Gel Precipitin Tests in Serodiagnosis of Human Coccidioidomycosis

John G. Ray, Jr,

JANUARY 1967

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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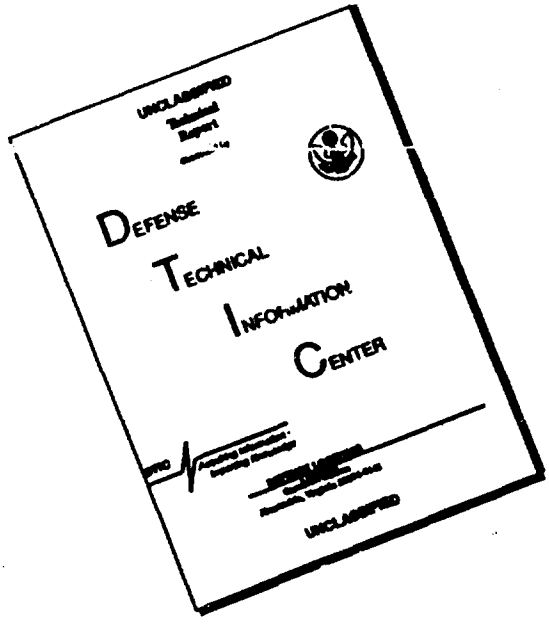

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Medical Investigation Division
MEDICAL SCIENCES LABORATORY

Project 1B622401A072

January 1967

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

The agar-gel precipitin-inhibition serological test for coccidioidomycosis was a more sensitive indicator of Coccidioides immitis antibodies than the tube precipitin, the agar-gel immunodiffusion, or the complement-fixation tests in assaying monkey sera, whether these sera were from prechallenge-vaccinated or postchallenged animals.

Generally, when applying this technique to a similar assay of human sera, an analogous finding persisted. However, a few human sera were positive by the complement-fixation test and negative by the agar-gel precipitin-inhibition test. These sera were diffused in agar-gel against various coccidioidin complement-fixation, tube precipitin, and agar-gel precipitin-inhibition test antigens with essentially negative results.

I. INTRODUCTION

In previous reports,[1,2] the agar-gel precipitin-inhibition (AGPI) and complement-fixation (CF) tests for coccidioidomycosis were compared for their ability to assay Coccidioides immitis antibodies in monkey and dog sera. In addition, the tube precipitin (TP) and the agar-gel immunodiffusion (AGID) tests[3] were compared with the above serological tests in one set of experiments.[2] The results indicated that the AGPI procedure was more sensitive than the CF, TP, or AGID tests in determining the C. immitis antibody content of these monkey sera, whether they were from prechallenge-vaccinated or postchallenged animals.

However, sufficient human serum specimens were not available at that time to perform an analogous assay. During the past year, a significant number of human coccidioidomycosis sera were obtained through the courtesy of Drs. Evelyn Wallraff, VA Hospital, Tucson; H. Gilbert Crecelius, Arizona State Department of Health; and John Bennett, NIH Clinical Center, to warrant such an assay. The results are reported here.

II. MATERIALS AND METHODS

An agar-gel medium previously described by Ray and Kadull,[4] a coccidioidin antigen prepared and supplied by Mr. John Converse, and a coccidioidomycosis antiserum, human or animal, of high CF titer (1:256 or greater) were employed in the AGPI test.

The CF titers in this report were attained by the source laboratory technique or by the Kolmer 100% fixation technique as used in our Fort Detrick Serology Laboratory (FDSL). The FDSL CF technique utilized the recommended Communicable Disease Center (CDC) CF antigen and human control antiserum.

III. RESULTS

Table 1 shows that the AGPI procedure detected titers in vaccinated and postchallenge monkey sera where there was no detectable CF titer. That these AGPI serum titers were detected earlier and persisted longer than those assayed by the CF technique is also evidenced in this table.

TABLE 1. COMPARISON OF SEROLOGICAL RESULTS IN MONKEY'S RESPONSE TO CUTANEOUS AND PULMONARY COCCIDIOIDOMYCOSIS[a]

Immunization		Prechallenge Titer 6 Months Postvaccination			15 Days After Respiratory Challenge with Silveira	
		Skin Test	CF Titer	AGPI Titer	CF Titer	AGPI Titer
Unvaccinated (controls)	T-40	-	-	-	-	-
	T-34	-	-	-	-	-
	T-74	-	-	-	-	-
	TR-74	-	-	-	-	-
	T-66	-	-	-	-	-
Viable vaccine (Silveira spores)						
dose 10	T-78	+	32	64	64	64
	S-22	+	-	8	8	64
	T-67	+	-	8	16	32
	T-44	+	-	4	8	32
dose 100	T-38	+	16	32	32	32
	T-30	+	-	4	8	16
	T-77	+	-	2	-	8
	T-57	+	-	4	8	16
dose 1000	T-63	+	8	32	8	32
	T-21	+	16	64	16	64
	T-17	+	-	16	-	32
	T-43	+	256	512	256	512
Nonviable vaccine (formalin-killed Cash strain)	S-38	±[b]	-	-	-	8
	S-13	±	-	-	-	4
	TR-76	±	-	-	-	2
	TR-75	±	-	-	-	32

a. Reciprocal titers are presented.
b. Questionable results (erythema but no induration).

When the AGPI test results were compared with those of the CF, TP, and AGID tests in an interlaboratory investigation of sera from immunized and nonimmunized postchallenged monkeys, the AGPI test proved more sensitive by detecting titers earlier, especially in the spherule vaccine group (Table 2).

In the combined cell fraction vaccine group, only the TP, AGID, and AGPI tests detected any antibody response in the S-49 monkey serum; the CF tests were negative. Antibodies to C. immitis in the K-20 serum of the intact spherule vaccine group were detectable only by the AGID and AGPI tests. It is also interesting to note in this vaccine group series that the Huppert CF test results either were equivalent in titer in the 2-, 8-, and 12-week serum samples or decreased in titer; the AGPI test indicated a decided increase in titer over these same time periods. Why was this so? There was a possibility that the two laboratories were measuring different antigen-antibody reactions.

Human sera obtained from the previously mentioned sources were compared by the AGPI and CF techniques. Initially, the samples obtained from Drs. Wallraff and Bennett were titrated with the results shown in Table 3.

The next group of human sera was obtained from Dr. H. Gilbert Crecelius and the titers were compared by the CF and AGPI test procedures (Table 4).

It is interesting to note that sera 17737, 17979, and 18961, having LBCF titers of 1:8, 1:4, and 1:32 respectively, were negative by the AGPI test. The clinical history revealed that serum 17737 came from a patient with a positive skin test, positive X-ray, and positive culture of C. immitis from bronchial washings; and serum 18961 came from a patient with a positive skin test, X-ray, and culture from animal inoculation. No case history was obtainable on serum 17979.

These sera were intriguing because now "the shoe was on the other foot" and I especially wished to know why this occurred in human sera when it was not apparent in monkey sera. Was this due to the difference in sera or to the difference in antigens that assay their antibody content?

TABLE 2. COMPARISON OF POSTCHALLENGE SEROLOGIC REACTIONS IN IMMUNIZED AND NONIMMUNIZED MONKEYS

Immunizing Antigen	Monkey	Tube Precipitin Test			AGID Test			AGPI Test			Complement-Fixation Test					
											Huppert			Ray		
		2 wk	8 wk	12 wk	2 wk	8 wk	12 wk	2 wk	8 wk	12 wk	2 wk	8 wk	12 wk	2 wk	8 wk	12 wk
Intact Spherule	K-21	0	0	0	±	+	+	32[b]	512	1024	8	0	2	20	0	80
	K-43	0	NS[a]	+	0	+	+	4	128	1024	0	8	8	NS	160	640
	K-11	0	0	+	0	+	+	4	128	256	0	8	4	NS	80	160
	K-20	0	0	0	0	+	+	0	128	1024	0	0	0	0	0	0
	P-17	0	0	0	0	+	+	0	32	256	0	AC[c]	128	0	80	640
Combined Cell Fraction	S-49	0	0	+	0	0	+	0	8	4	0	0	0	0	0	0
	K-45	0	0	+	0	+	+	0	512	1024	0	8	512	AC	80	1280
	P-12	0	0	+	0	+	+	0	256	1024	0	16	256	0	640	640
	P-14	0	0	+	0	+	+	0	128	1024	0	4	32	0	320	320
	P-15	0	0	0	0	0	+	0	16	8	0	0	2	0	10	10

a. No sample.
b. Reciprocal of tube dilution.
c. Anticomplementary.

TABLE 3. COMPARATIVE HUMAN COCCIDIOIDOMYCOSIS SERUM TITRATIONS

Serum	Source CF Titer[a]	FDSL CF Titer	AGPI Titer
Gephard	Neg	Neg	2
Hodge	8	10	32
Pavlok	Neg	Neg	Neg
Sukey			
7-14-65	Neg	40	32
7-17-65	Neg	40	32
7-22-65	Neg	20	16
C. Lee			
9-22-64		40	256
10-13-64	16	40	128
11-24-64		20	64
12-1-64	32	20	64
2-23-65	16	20	32
R. Capobres			
7-14-64		80	256
8-18-64		80	256
9-15-64		80	128
10-6-64		80	128
11-24-64		40	64
8-24-65		40	64
1-4-66		20	16
2-8-66		20	32
CDC Lot 6 Human Control Serum	64	80	128

a. Reciprocal titers are recorded.

TABLE 4. COMPARATIVE HUMAN COCCIDIOIDOMYCOSIS SERUM TITRATIONS

Serum	Reciprocal Titers		
	Source LBCF	FDSL CF	AGPI
17202	16	Neg	32
17204	64	10	128
17206	64	80	128
17338	64	40	128
17729	32	10	128
17730	64	40	128
17731	128	40	256
17737	8	Neg	Neg
17843	8	Neg	32
17848	8	Neg	16
17973	16	10	16
17979	4	Neg[a/]	Neg
18113	8	5	16
18184	16	10	16
18185	8	5	32
18799	16	10	64
18961	32	Neg[a/]	Neg

a. Anticomplementary serum.

Each of these sera was diffused in agar-gel against several antigen preparations. Dr. Huppert supplied four antigens: the XV-B-60F, UFR coccidioidomycosis CF antigen; the XV-B-60L coccidioidomycosis precipitin antigen; the XV-B-60L IDTP antigen; and the XV-B-60F, UFR IDCF antigen. In addition, Lot 8 CF antigen was obtained from the Communicable Disease Center (CDC), and Lot 3 coccidioidin was obtained from Mr. John Converse. These six antigens were employed in the agar-gel double diffusion of the above sera as well as of a positive control serum, 17730, from Dr. Crecelius' laboratory, and Lot 6 CDC human positive CF control serum. Each reagent was placed in its respective well every 24 hours for 5 days. After the initial 5-day aerial recharging with the reagents in their wells, the wells were recharged 24 hours before the plate reactions were to be photographed. This treatment would tend to negate differences in concentration of the antigen or antibody content in these reagents.

Figure 1 depicts the serial diffusion of these reagents and shows that no activity was forthcoming from sera 17979 and 17737 (both negative by the AGPI technique, positive by the LBCF method. Figure 2 shows that the positive control serum 17730 and Lot 6 CDC CF human serum control were both positive to varying degrees with the diffused antigens.

After continuing this procedure for 18 and 21 days (Figure 3) there appeared a weak antigen-antibody reaction between the two sera 17737 and 17979 and the Lot 3 AGPI and the XV-B-60F (UFR coccidioidomycosis CF) antigens. To determine what this measured antigen-antibody reaction was related to would require a coccidioidomycotic test antigen-antibody evaluation.

This experiment, however, did determine that use of these antigens in their standard test concentrations would not show an antibody titer to coccidioidomycosis with these negative sera, either by inhibition of the antigen in the AGPI test, or by binding of complement in the CF test. Similarly, AGID antigens would not have detected the presence of coccidioidomycosis antibodies in these sera, because a larger total concentration was employed in both reagent wells and a longer observation period was used in this experiment than is used in the standard 48- to 72-hour incubation observation period. However, certain CF test antigens were measuring an apparently different antibody content in suspect coccidioidomycotic sera because of their differences in antigenic content. This certainly requires further investigation.

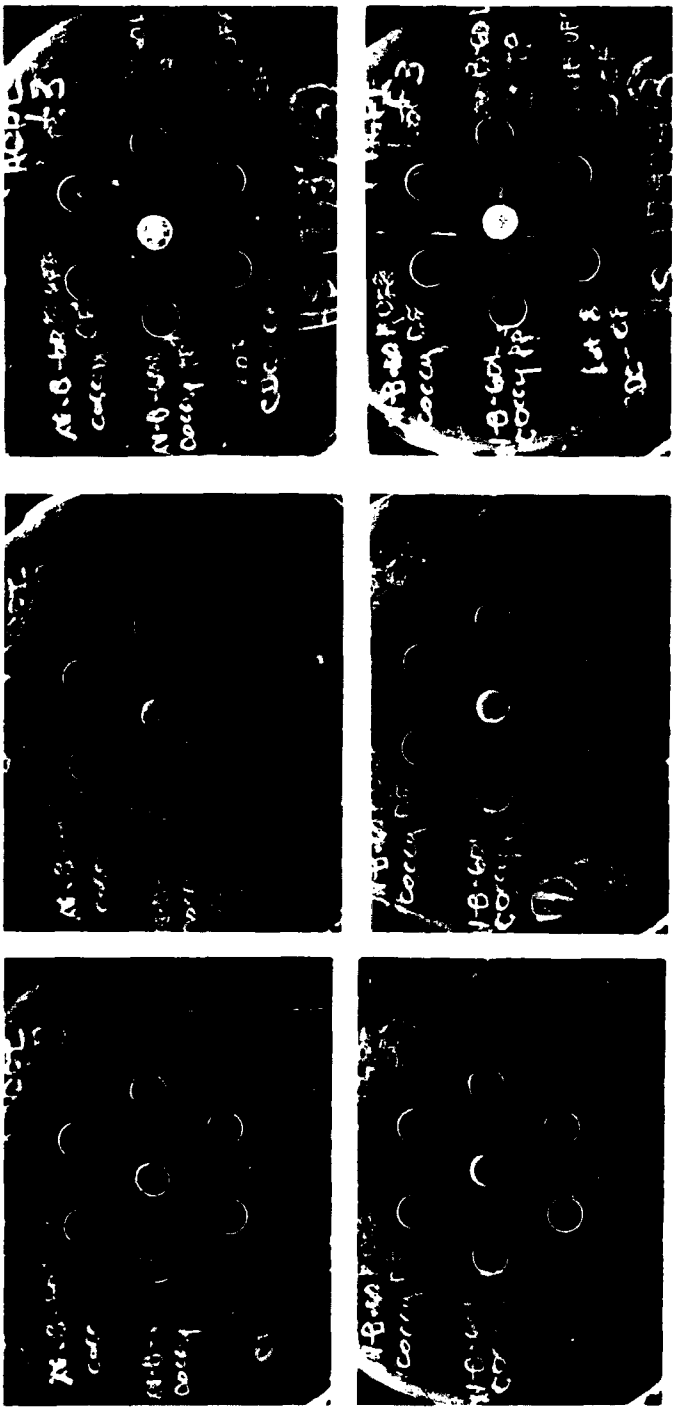

Figure 1. Serial Double Diffusion of AGPI Negative Coccidioidomycosis Human Sera with Selected CF, TP, AGID, and AGPI Test Antigens.

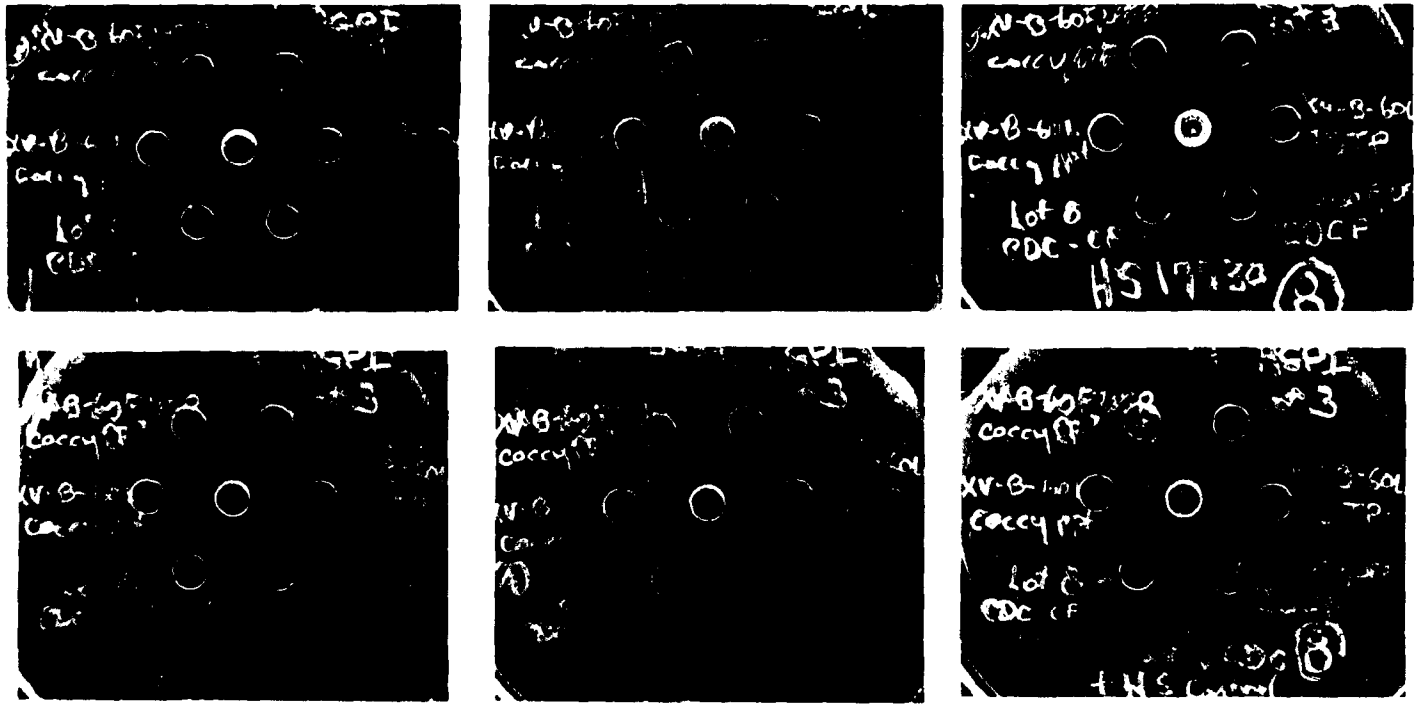

Figure 2. Serial Double Diffusion of AGPI Positive Coccidioidomycosis Human Sera with Selected CF, TP, AGID, and AGPI Test Antigens.

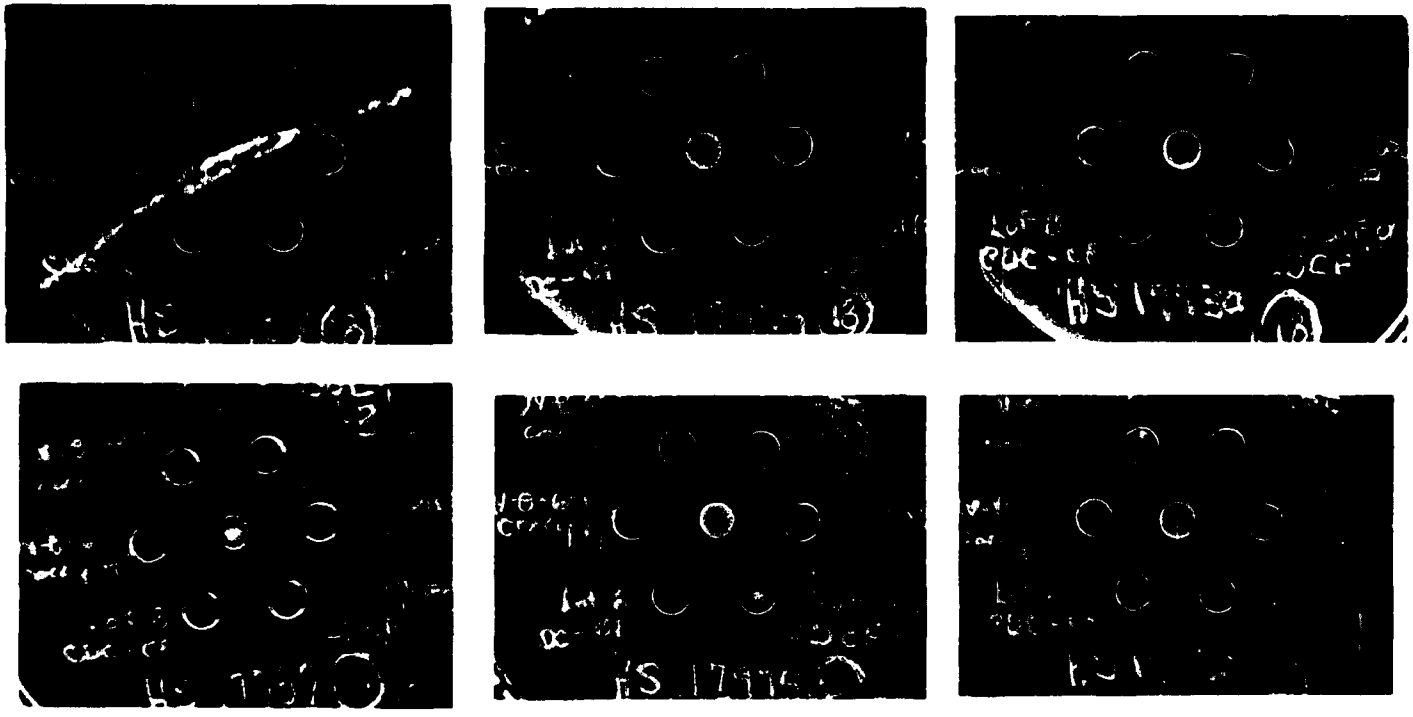

Figure 3. Continued Serial Double Diffusion of AGPI Negative and Positive Coccidioidomycosis Human Sera with Selected CF, TP, AGID, and AGPI Test Antigens.

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14. Key Words

*Agar-gel precipitin inhibition technique
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